



Figure S2. See legend over page

Figure S2 NF90 Controls DICER Expression by Modulating Association of Microprocessor with miR-3173 and Efficiency of DICER Pre-mRNA Splicing. **(A, B)** NF90 regulates DICER expression level. Extracts of 293T cells transfected with siRNAs targeting NF90/NF110 **(A)**, NF90 **(B)** or a non-targeting control (Scr) were analyzed by immunoblot using the antibodies indicated. **(C)** Extracts of control or NF90/NF110 knock-down 293T cells were analyzed by RT-qPCR using PCR primers amplifying spliced or unspliced transcripts. Values obtained for the control sample (siScr) were attributed a value of 1 (mean \pm SEM obtained from 3 independent experiments, (** $P < 0.01$, * $P < 0.05$, independent Student t test). **(D)** Extracts of control or NF90/NF110 knock-down 293T cells were analyzed by RT-qPCR using PCR primers specifically amplifying spliced or unspliced CTDSPL or CTDSP1 transcripts, as indicated. The splicing efficiency was calculated by the ratio of spliced to unspliced transcripts. Values obtained for the control sample (siScr) were attributed a value of 1. Data represent mean \pm SEM obtained from 3 independent experiments (ns = not significant, independent Student t test). **(E)** NF90/NF110 does not affect RNAPII association with the promoter-proximal region of Dicer. 293T cells transfected with control or NF90/NF110-specific siRNA were analyzed by ChIP using antibody to RNAPII or a control IgG as indicated. Immunoprecipitates were analyzed by qPCR using primers to amplify the region proximal to the TSS of Dicer. Data represent mean \pm SEM obtained from 3 independent experiments (ns = not significant, independent Student t test). **(F)** Recombinant NF90 and DGCR8 dsRBDs used in RNA EMSA were analyzed by SDS-PAGE and Coomassie brilliant blue staining (CBB). Samples were analysed in parallel by immunoblot using the anti-NF90 antibody (IB anti-NF90) that was used in EMSA. **(G)** RNA EMSA was performed with increasing concentrations of rNF90 indicated on the figure together with either pri-miR-3173 or pri-miR-21 probes as indicated. **(H)** Schematic showing the cleavage site within miR-3173 stem loop as mapped by 5' RLM-RACE. PCR products shown in Figure 2H were sequenced to identify the 5' extremity corresponding to the RNA cleavage site augmented in the absence of NF90/NF110. Nts shown in red correspond to the 3' end of miR-3173-3p. Red and green arrowheads indicate the cleavage sites identified by sequencing of PCR products formed using For and Rev1 primers and For and Rev2 primers, respectively.